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THE HAPLOIMMUNOSTORM SYNDROME: A DISTINCT CLINICAL ENTITY SEEN IN HLA-MISMATCHED CELLULAR IMMUNOTHERAPY

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We have observed a new infusion related clinical entity named haploimmunostorm (HIS), observed after minimal immunosuppressive HLA-3/6 or 4/6 mismatched stem cell transplantation. We have performed a total of 44 HLA-mismatched transplants with escalation of the $CD3^+$ dose from 1×10^6-2×10^8 cells/kg using G-CSF primed PBSC, with a conditioning regimen of 100 cGy TBI. The $CD34^+$ dose was $>2 \times 10^6$ cells/kg. This post-infusion HIS syndrome occurred in 26 out of 30 (87%) patients with a $CD3^+$ dose more than 1×10^8 cells/kg. In the HIS syndrome, a constellation of symptoms occurred, some with variable penetrance, in which hyperpyrexia and malaise were a constant feature occurring as early as four hours after cell infusion with a median time of 14-hours. A morbilliform rash was seen in 40% of patients. Biopsies of these rashes revealed no evidence of hyperacute or acute graft versus host disease; rather epidermal spongiosis with lymphocytic invasion was usually seen. Diarrhea was present in a smaller subpopulation of patients (20%) and biopsies taken of the colon also failed to show any evidence of acute graft versus host disease. Transient elevations of liver enzymes occurred in 40% of the patients within 6–24 hours after infusion. Hematologic manifestations consisted of marked lymphopenia, which began at the time of initial hyperpyrexia. Steroids were used successfully if the HIS syndrome lasted more than 72 hrs. Preliminary cytokine level analysis showed variable increases of serum TNF-alpha and IL-2 post bone marrow transplantation compared with pre-transplant levels. Complete cytokine level analysis is ongoing. This syndrome is believed to be immunologically based and represents neither hyperacute nor acute graft versus host disease. This syndrome is different than an engraftment syndrome reported in some patients undergoing autologous hematopoietic stem cell transplant, particularly patients with breast cancer. Engraftment syndrome occurs at the time of engraftment, opposed to HIS in which there is transient chimerism lasting on average a week after cell infusion. Engraftment syndrome is similar to HIS in that it is associated with fever and rash but deviates with presence of capillary leak and pulmonary infiltrates.

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EVALUATION OF THE STRUCTURAL BASIS OF T CELL ALLORECOGNITION USING RECOMBINANT HLA CLASS I MULTIMERS

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Graft-versus-Host disease is a major complication of any stem cell transplant that uses stem cells from an HLA-mismatched donor. However, these transplants may also possess potential for beneficial non-self HLA restricted responses to tumor antigens. Manipulation of these alloreactivities requires information on the structural basis of T cell allorecognition. In the $CD8^+$ T cell response to allogeneic HLA class I molecules both peptide-independent and peptide-specific recognition have been suggested to play a role. We examined peptide-specific allorecognition using A*0201 tetramers representing five self-peptides known to bind endogenously to HLA-A*0201. Small numbers of $CD8^+$ T cells specific for these self-peptides were detected within four in vitro stimulated T cell populations specific for allogeneic HLA-A*0201. Peptide-independent allorecognition was investigated using artificial antigen presenting constructs (aAPCs) comprising latex beads coated with single A*0201/peptide complexes, CD54 and CD80. The aAPCs stimulated production of IFN-γ by T cells specific for the A*0201/peptide complex on the bead-surface. None of the four T cell populations specific for allogeneic HLA-A*0201 were stimulated by aAPCs whereas they did produce IFN-γ when stimulated with cells naturally expressing HLA-A*0201. The results indicate that alloreactive populations comprise subsets of T cells specific for self-peptides presented by non-self HLA class I molecules with no evidence of a peptide independent component.

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SILENCING HUMAN NKG2D, DAP10 AND DAP12 REDUCES CYTOTOXICITY OF *IN VIVO* ACTIVATED $CD8^+$ T AND NK CELLS

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NKG2D is a cell surface receptor expressed on NK cells, $CD8^+$ T cells, γδ T cells. NKG2D is activated by stress-induced ligands MICA and MICB, and UL16 binding proteins which are expressed on target cells modified by infection, or stress. Binding of NKG2D on NK cells results in an activation signal that bypasses or overrides inhibitory signals from major histocompatibility complex (MHC) I resulting in cytotoxic granule content release and target cell killing. Signaling is achieved by selective expression of the adaptor proteins DAP10 and DAP12. We have shown that $CD8^+$ T cells activated with IFN-γ, anti CD3 mAb and IL2 express NKG2D and share functional and phenotypic properties with NK cells. Activated $CD8^+$ T cells up-regulate NKG2D as well as DAP10 and DAP12 during ex-vivo activation and expansion which correlates with cytotoxic function. To further elucidate the role of NKG2D signaling in $CD8^+$ T cell-mediated cytotoxicity, 21-nucleotide small interfering RNAs (siRNA) were designed the NKG2D gene. FACS analysis, northern and western blots demonstrated that effector cells transfected with siRNA incorporated into the pLV ThM lentiviral vector resulted in >90% suppression of NKG2D expression after 72 hrs. In both $CD8^+$ T cells and NK cells, suppression of NKG2D resulted in a >90% decrease in cytotoxicity against tumor cell line targets (RPMI and U266). We also performed experiments using an autologous tumor targets derived from ascites. Similar to the results obtained using $CD8^+$ T cells and NK cells against target cell lines, suppressing NKG2D expression in activated $CD8^+$ T Cells resulted in >90% decrease in cytotoxicity against the autologous tumor targets. To characterize the role of DAP10 and DAP12 in NKG2D cytotoxic signaling, we generated siRNA spanning DAP10 and siRNA for DAP12. Using northern blot analysis, >90% suppression of DAP 10 and DAP12 was observed when $CD8^+$ T cells and NK cells were treated with the siRNA. Suppression of DAP10 expression in $CD8^+$ T cells and NK cells resulted in a decrease in cytotoxicity of more than ~75% compared to control cells. Suppressing DAP12 expression also resulted in significant reduction in cytotoxicity, however was consistently less than that of DAP10. When DAP10 and DAP12 were simultaneously silenced in $CD8^+$ T and NK cells, an ~82% reduction in cytotoxicity was observed. These that both DAP12 and DAP12 are involved in activated and expanded $CD8^+$ T cell cytotoxicity.

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CD6-DEPLETED MOBILIZED STEM CELLS FOR MODIFICATION OF HVG AND GVH REACTIONS AFTER HLA-HAPLOIDENTICAL MARROW TRANSPLANTATION

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Allogeneic stem cell transplantation is limited to patients with a histocompatible donor, but for patients with advanced acute leukemia and high-grade lymphoma HLA-haploidentical transplantation may be considered. Rejection of the transplant and graft-versus-host disease are major obstacles and T-cell depletion eliminates the graft-versus-leukemia effect and causes prolonged immune deficiency. Here we studied the use of CD6-depleted G-CSF mobilized blood cells (mbc) 6 days after transplantation of unmodified marrow in order to suppress host-versus-graft and